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Evaluation of Performance Characteristics of Automated Measurement Systems for Allergy Testing

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Summary: Reliability of test results, convenient handling and flexibility are major requirements on automated immunoassays systems. To investigate to what extent these requirements were met by the Pharmacia CAP and DPC IMMULITE and DPC Microplate Systems, we evaluated several performance characteristics of assays of specific IgE against some common inhalant allergens as well as the atopy tests Phadiatop (Pharmacia CAP System) and AlaTOP (DPC IMMULITE and Microplate System).

Comparing Phadiatop and AlaTOP results (n = 95) to clinical data, the sensitivity was found to be 97% in the Pharmacia CAP System and 82% in the AlaTOP-DPC Microplate System and 88% with AlaTOP-IMMULITE. Specificity was in all cases higher than 90%.

The pooled total variation was more than twice as high with the DPC Microplate System as compared to the Pharmacia CAP System in our first investigation. A second investigation showed similar values.

The investigation of systematic differences showed that the error contribution of sample related differences between the systems was even larger and far exceeded the analytical variation. Thus the two methods do not seem to be measuring the same specific IgE antibodies. In 8 out of 8 cases with the Pharmacia CAP System positive and DPC negative results and in 2 out of 2 cases with DPC positive and Pharmacia CAP System negative results, the presence of IgE antibodies could be confirmed by IgE immunoblotting. Serum dilutions showed very irregular O/E patterns for the DPC Microplate System.

There was no effect when adding non-specific IgE to serum samples. Addition of competing IgG antibodies showed a moderate decrease in binding of specific IgE in the Pharmacia CAP System when increasing amounts of IgG were added. The effect in the DPC Microplate System was more pronounced with large decreases, or increases of measured values even at lower concentration of the competing antibody. The results may indicate insufficient allergen concentration in the DPC assay and draw attention to the risk for undesirable complex formation between allergen and antibody in solution.

The combination of the two DPC systems did not offer any advantages over Pharmacia CAP System from the handling or work flow point of view.

Introduction

Automated systems in our laboratories are expected to be easy to handle and as flexible as possible to enable laboratory personnel to work with them at all times. The recently introduced IMMULITE (DPC) (1, 2) which includes the test AlaTOP allows random access allergy screening testing and would reduce pre analytical work time.

AlaTOP IMMULITE and follow up with allergen specific IgE tests on a DPC Microplate automated micro-

plate system (DPC) could be an alternative to the Pharmacia CAP System presently used in one of our laboratories for differential as well as specific allergy testing in the following configuration: RoboCAP, AutoCAP and FluoroCount 96. Studies performed previously (3, 4) usually compared performance to the skin prick test (SPT). In this study we wanted to examine the performance of both systems from a quantitative standpoint.

The need for initial differential tests is indicated in several studies (5, 6, 7). The quantitative measurement of specific IgE antibodies has been proposed as a basis to correlate severity of disease with serum concentration of IgE antibodies (8, 9). This has also been expressed as a preference by the clinicians at and around Maaslandsziekenhuis, Sittard. The aim of the study was to investigate the performance characteristics of the tests run on the combination of two DPC systems as compared to Phadiatop and specific IgE antibody assay on the Pharmacia CAP System.

The following performance characteristics were evaluated:

- clinical sensitivity and specificity of AlaTOP in the two DPC systems and of Phadiatop®
- within, between and total assay run precision
- systematic differences between the specific IgE assays
- consistency after dilution
- interference from allergen specific IgG antibodies
- influence from unspecific IgE.

Furthermore the systems were evaluated with respect to user friendliness.

Materials and Methods

Test technologies

The Pharmacia CAP System FEIA is a fluorescent enzyme immunoassay. The allergens, covalently coupled to the ImmunoCAP, react with the specific IgE in the patient serum and enzyme labelled anti-IgE antibodies are added to form a complex. This is incubated with a development agent. When the reaction is finished the fluorescence is measured.

The DPC Microplate System is an enzyme immunometric assay based on liquid ligand labelled allergens and separation by ligand-coated wells. The specific IgE in the patient sample forms an allergen-IgE complex which is incubated with a multivalent anti-ligand which in turn links the allergen-IgE complexes and the ligand-coated wells. Horseradish peroxidase labelled monoclonal anti-IgE antibodies are added to the allergen-IgE complex. A chromogenic indicator is added and the result is measured kinetically.

IMMULITE is a chemiluminescent enzyme immunoassay, based on the same principle as Microplate but utilising an alkaline phosphatase-labelled anti-human IgE which reacts with a chemiluminescent substrate. Anti ligand coated polystyrene beads are used to capture the ligand labelled allergens.

WHO-IgE based calibrators are used for determination of total IgE and values expressed in kU/l. In Pharmacia CAP System these standards are also used for the determination of specific IgE antibodies and the values are expressed in kU_A/l. For study purposes we have used kU/l as the measuring unit for both systems. For further details of the different test systems, see the directions for use from the system suppliers.

Instruments and reagents

Pharmacia CAP System instruments and reagents

RoboCAP:	Pipetting of sera and allergen distribution
AutoCAP:	Incubation, reagent addition, washing and elution
FluoroCount 96:	Measurement of fluorescence
MasterCAP:	Patient, assay and instrument management, evaluation and report
Pharmacia CAP System	Specific IgE and Phadiatop

DPC instruments and reagents

DPC Microplate EIA System:	Measurement of absorbance
MARK 5 Pipettor:	Sera and reagent pipetting
Microplate MAX Plate Processor:	Washing and incubation
MAX software:	Patient, assay and instrument management, evaluation and report
DPC Microplate System	Specific IgE and AlaTOP

IMMULITE

IMMULITE	Incubation, process, reagent addition, measurement and evaluation
IMMULITE	AlaTOP allergy screen

Specific IgE allergens

1d, House dust mite	g6, Timothy, *Phleum pratense*
e1, Cat dander	t3, Common silver birch, *Betula pendula*
e5, Dog dander	w6, Mugwort, *Artemisia vulgaris*

Immunoblotting

Pharmacia Diagnostics	PAGE immunoblotting was performed essentially as described by *Bengtsson* et al. (10). The scanning of the IgE-antibody zones of the blot was performed with the help of Image Master™, Pharmacia Biotech, Uppsala Sweden.
DPC	Western Blot procedure was performed essentially as described by *U. K. Laemmli* (11).

Statistical Methods

Evaluation of precision

The standard deviations of the error components within and between runs were estimated from analysis of variance and are presented as coefficients of variation, CV(%), i. e. the standard devia-

tion in per cent of the mean. As an estimate of a common CV for all species, the pooled CV is calculated according to

$$CV_{Pooled} = \sqrt{\frac{CV_{d1}^2 + CV_{e1}^2 + CV_{e5}^2 + CV_{g6}^2 + CV_{t3}^2 + CV_{w6}^2}{6}}$$

Comparison of methods

The results from the method comparison are presented graphically in xy-plots. Straight lines have been fitted to the xy-plots according to the method by *Passing & Bablok* (12–14). As pointed out by *Altman & Bland* (15), it may be more relevant to consider the differences or ratios between the methods and therefore ratio plots, y/x against (x + y)/2, are also presented. By applying the statistical analysis suggested by *Nilsson* (16) the existence of sample-related differences can be tested and their standard deviations estimated. To facilitate the interpretation the standard deviation of the sample-related differences is expressed in relation to the expected contribution to the scatter from the random variation within runs, i.e. by the quotient between the two standard deviations, in this paper denoted as the coefficients of sample-related disagreement, CSD. This coefficient gives the relative importance between sample-related differences and the variation within runs when results from the two methods are compared. A value of CSD > 0.75 corresponds to the rule of thumb given in l. c. (16) for rejection of the hypothesis of no sample-related differences (with a significance level of 5% or less). A CSD > 1.5 should be considered an indication of a crucial contribution to a disagreement between the methods from sample-related differences.

As relative differences are more relevant than absolute ones, the statistical analysis is performed after logarithmic transformation of the concentrations.

Experimental Design

Estimation of clinical sensitivity and specificity of AlaTOP and Phadiatop

Serum samples from 100 consecutive patients with suspected allergy were used to assess clinical sensitivity and clinical specificity. 34 were clinically verified as atopic and 61 as non-atopic, while in 5 cases, clinical history and available in vivo test results were inconclusive. The latter were excluded from the comparison. The following alternative criteria were used for positive diagnosis of atopy; SPT of 3+ (equal to wheal of histamine control, 10 g/l), SPT 2+ in combination with positive case history for the same allergen, SPT 1+ or 2+ in combination with positive RAST for the same allergen or a positive provocation test. The criteria for a negative diagnosis of atopy was; SPT negative, SPT 1+ in combination with negative case history or negative RAST or negative provocation test. Patients not fulfilling criteria for positive or negative atopy diagnosis were considered as inconclusive.

Age:	Average 28 years, range 17–67 years
Sex:	60 females and 40 males
Diagnosis:	41 Bronchial asthma, 19 Seasonal rhinitis and 63 Perennial rhinitis.

The serum samples were tested with Phadiatop (Pharmacia CAP System) and AlaTOP (IMMULITE and DPC Microplate System).

Evaluation and comparison of performance characteristics for measurement of specific IgE antibodies

All assays were allocated to five runs consisting of two plates and evaluated with a separate calibration curve and performed during a five day period. All samples were coded and assayed blind.

Precision experiment

Allergen specific IgE antibodies against the allergens d1, e1, e5, g6, t3 and w6 were assayed in one sample per allergen in five replicates in each of the five runs.

Evaluation of systematic differences

62 patient sera per allergen (d1, e1, e5, g6, t3 and w6) were selected according to the results with our current routine method (Pharmacia CAP System) and used to assess the possible concordance between DPC Microplate system specific IgE Microplate (DPC Microplate System) and RAST (Pharmacia CAP System).

Dilution experiments

Two patient sera for each of the allergens d1, e1, e5, g6, t3 and w6 were diluted and assayed in duplicate, undiluted and diluted with negative serum to 1 : 2, 1 : 4, 1 : 8 and 1 : 16. The diluent consisted of pooled human serum from healthy controls, presenting responses below 50% of the cut-off in both systems and a concentration of total IgE of approximately 5 kU/l. All dilutions were made independently from the origin sample. All assays were allocated to the same run. The 100 kU/l calibrator was also diluted and assayed in the same way for the two systems, respectively.

Addition experiment: IgE

One negative control serum per allergen (d1, e1, e5, g6, t3 and w6) and method was spiked with myeloma IgE to total IgE levels of approximately 1000 and 3000 (kU/l) units to test for unspecified binding of IgE protein. All samples were assayed in duplicate in the same run.

Addition experiment: Allergen specific IgG antibodies

Allergen specific rabbit antisera were diluted with normal rabbit serum to 1 : 2, 1 : 4 and 1 : 8. One patient serum for each of the allergens d1, g6 and t3 was mixed in equal parts with negative human serum, normal rabbit serum, the undiluted and the three dilutions of the anti sera. Thus a series of six samples with the same proportion of the patient serum is obtained. One is diluted by human negative serum and the other five contain the IgG antibodies (rabbit antiserum) in the proportions 1 : 2, 1 : 4, 1 : 8, 1 : 16 and 0. All six samples for each allergen were assayed in duplicate in the same run. This experiment shows relative differences in risk for interference by non-IgE antibodies competing for the same allergen and may be considered as a test of the capacity of the allergen reagent.

Comparison of calibrators

The assigned values of the calibrators were checked for both systems by assaying the DPC Microplate System calibrators in duplicate in the Pharmacia CAP System and vice versa.

Results and Discussion

Clinical performance of Phadiatop and AlaTOP

The results for Phadiotop (Pharmacia CAP System) and AlaTOP (IMMULITE and DPC Microplate System) are

Tab. 1 Clinical sensitivity and specificity for Phadiatop and AlaTOP.

Diagnosis	Number	Phadiatop		AlaTOP IMMULITE		AlaTOP Microplate	
		pos	neg	pos	neg	pos	neg
Atopic	34	33	1	30	4	28	6
Non-atopic	61	4	57	0	61	4	57
Sensitivity (%)		97		88		82	
Specificity (%)		93		100		93	

given in table 1 together with estimates of clinical sensitivity and specificity.

33 out of 34 sera (97%) of the atopic patients were correctly classified as positive by Phadiatop. The corresponding figures for AlaTOP were 30 out 34 for IMMULITE and 28 out of 34 for DPC Microplate System. Specificity varied from 93–100%. These findings correlate well with results reported in other studies (17–19). A differential diagnostic test for atopy is often the first test to be performed on a patient and is followed by allergen specific testing in the cases considered atopic i. e. in those cases with positive Phadiatop/AlaTOP. It is obvious that high sensitivity is essential in this situation.

Technical performance of specific IgE test systems

Precision

The random variation expressed as total CV as well as the variation within and between runs for the DPC Microplate System were more than twice as high as for the Pharmacia CAP System (see tab. 2, trial 1). The obtained results (kU/l) in trial 1 were also higher with the Pharmacia CAP System than with the DPC Microplate System, apart from g6 where the figure was obtained by extrapolation by the DPC software.

In order to rule out a faulty instrument as the cause of imprecision (tab. 2, trial 4 only DPC) and to compare the precision in the lower part of the measuring range

Tab. 2 Results from first (trial 1) and second (trial 2 & 3) precision experiment. Mean concentrations from 5 runs are given in kU/l except trial 1 with DPC which is based on 10 runs. Coefficients of variation, CV, are given in %. * = Value obtained by extrapolation

Allergen	Estimate	Pharmacia CAP System			DPC Microplate System			
		trial 1	trial 2	trial 3	trial 1	trial 2	trial 3	trial 4
d1	Mean conc. kU/l	10.1	0.58	1.70	5.2	0.36	1.20	38.7
	Between run CV (%)	7.5	7.2	5.3	17.6	10.4	8.6	19.3
	Within run CV (%)	6.3	9.4	9.2	17.3	12.5	15.5	31.3
	Total CV (%)	9.8	11.8	10.6	24.7	16.2	17.7	35.9
e1	Mean conc. kU/l	23.5	0.63	2.25	15.4	1.70	3.23	6.3
	Between run CV (%)	6.5	6.3	6.0	10.8	9.0	7.0	6.8
	Within run CV (%)	9.8	16.0	6.2	12.3	10.9	10.5	9.0
	Total CV (%)	11.6	17.2	8.6	16.3	14.1	12.6	11.3
e5	Mean conc. kU/l	27.8	0.44	2.28	27.3	0.37	1.85	94.5
	Between-run CV (%)	6.3	9.0	5.5	16.5	5.5	9.4	13.4
	Within run CV (%)	7.5	8.7	8.0	30.0	14.0	14.5	43.9
	Total CV (%)	9.8	12.5	9.7	34.2	15.0	17.2	45.8
g6	Mean conc. kU/l	72.1	0.45	2.16	228.8*	0.096*	1.76	14.1
	Between run CV (%)	4.8	6.8	5.1	24.2	10.4	8.8	1.76
	Within run CV (%)	12.1	7.3	6.8	22.1	9.4	11.6	7.29
	Total CV (%)	13.0	10.0	8.5	32.8	14.1	14.6	7.50
t3	Mean conc. kU/l	9.6	0.57	1.39	6.0	0.37	1.85	35.7
	Between run CV (%)	5.9	9.5	4.8	14.4	5.5	9.4	11.3
	Within run CV (%)	4.3	7.5	7.6	16.4	14.0	14.5	20.3
	Total CV (%)	7.3	12.2	8.9	21.9	15.0	17.2	23.2
w6	Mean conc. kU/l	7.7	0.53	–	1.8	0.92	–	0.25*
	Between run CV (%)	5.3	5.8	–	9.4	16.7	–	7.4
	Within run CV (%)	6.1	7.7	–	19.6	12.7	–	15.1
	Total CV (%)	8.1	9.6	–	21.8	21.0	–	16.9
Pooled CV	Between run CV (%)	6.1	–	–	16.2	–	–	–
	Within run CV (%)	8.1	–	–	20.4	–	–	–
	Total CV (%)	10.2	–	–	26.1	–	–	–

(tab. 2, trial 2, 3) a second investigation was performed with the Pharmacia CAP System and manually (without Mark V pipette) with the DPC System. This showed a similar relation between the systems in terms of coefficient of variation as in trial 1. In trial two, three and four, new sets of sera were used, so no correlation between the trials was possible.

The Mark V instrument might contribute to a general increase of the imprecision, but random variation of the size shown by the DPC Microplate System makes testing with single determinations questionable.

Systematic differences

The calibrator range for both methods is 0.35–100 kU/l. The distribution of results below, within and above the measurement range with the two methods is given in table 3.

A high number of samples, between 4% and 16% for the different species, were reported as above the highest standard point in the DPC Microplate System. The reason for this can not be deducted from this experiment but the high variation, the unpredictable behaviour of serum samples and the calibrator in the dilution experiments and the need for individual optimisation of the allergen reagents are probably major contributing factors.

A first comparison between methods was performed on a class basis and showed the agreement between negative samples. 7% (12 out of 197) of the samples were positive in the Pharmacia CAP System and negative in the DPC Microplate System. Out of the positive samples (n = 155), we observed that 45% were within the same class. 34% of the positive samples in DPC were within ± 1 class of Pharmacia values, 19% of the positive sample in DPC within ± 2 classes of Pharmacia values and 2% deviated more than 2 classes from the Pharmacia value. A connected observation was that the DPC Microplate System gave lower values than Pharmacia in the low range and higher values in the high range. This means that of those results matching (n = 75), 72 results were found within class 2–4.

Tab. 3 Distribution of results below, within and above the calibrator range (0.35–100 kU/l). Shadowed boxes show discrepancies between the systems.

Allergens	Pharmacia CAP System	DPC Microplate		
		Below	Within	Above
d1	Below	32	0	0
	Within	5	22	3
	Above	0	0	0
e1	Below	31	1	0
	Within	2	22	5
	Above	0	1	0
e5	Below	31	0	0
	Within	0	24	7
	Above	0	0	0
g6	Below	31	1	0
	Within	3	19	7
	Above	0	0	1
t3	Below	29	1	0
	Within	0	22	10
	Above	0	0	0
w6	Below	31	1	0
	Within	2	25	3
	Above	0	0	0

Cases with discrepant results in the low range (tab. 3 and fig. 1), i.e. measurable with the Pharmacia CAP System but not with the DPC Microplate System and vice versa, were further studied by immunoblotting.

The twelve samples which were Pharmacia positive and DPC negative had specific IgE values in the range of 0.75–12.1 kU/l, eight of these were still available and sent to Pharmacia Diagnostics for analysis. In seven of eight cases presence of allergen specific antibodies could be demonstrated and optical density of blotting pattern scanned, see figure 1. The IgE antibodies were found to represent major as well as minor allergenic components of house dust mite, cat dander, timothy pollen and mugwort pollen (Pharmacia, personal communication). In the remaining case, serum no. 80, not present in figure 1, very week staining corresponding to the major mugwort allergens could be seen but not recorded because of limitations of sensitivity in the scanning equipment. All the cases with positive Pharmacia CAP System readings were confirmed by the analysis of IgE antibodies against individual allergenic components.

Two sera, no. 174 and no. 267, resulted in DPC positive and Pharmacia negative results. IgE antibodies detectable in the Western blot procedure were reported for timothy pollen, birch pollen, mugwort pollen and cat epithelia. No immunoblotting figures were included in the communication (DPC, personal communication).

Quantitative evaluation of systematic differences

For investigation of quantitative agreement between the methods both plots of y (DPC) versus x (Pharmacia) and of y/x (on a logarithmic scale) versus (x + y)/2 are given in figure 2. The estimated straight lines and coefficients of correlation for the xy-plots according to *Passing & Bablock* are given in table 4. Overall there is a low correlation between the methods. In order to examine the disagreement between the methods the suggestions put forward by *Nilsson* (16) were followed. Only samples with both results within the calibrator range

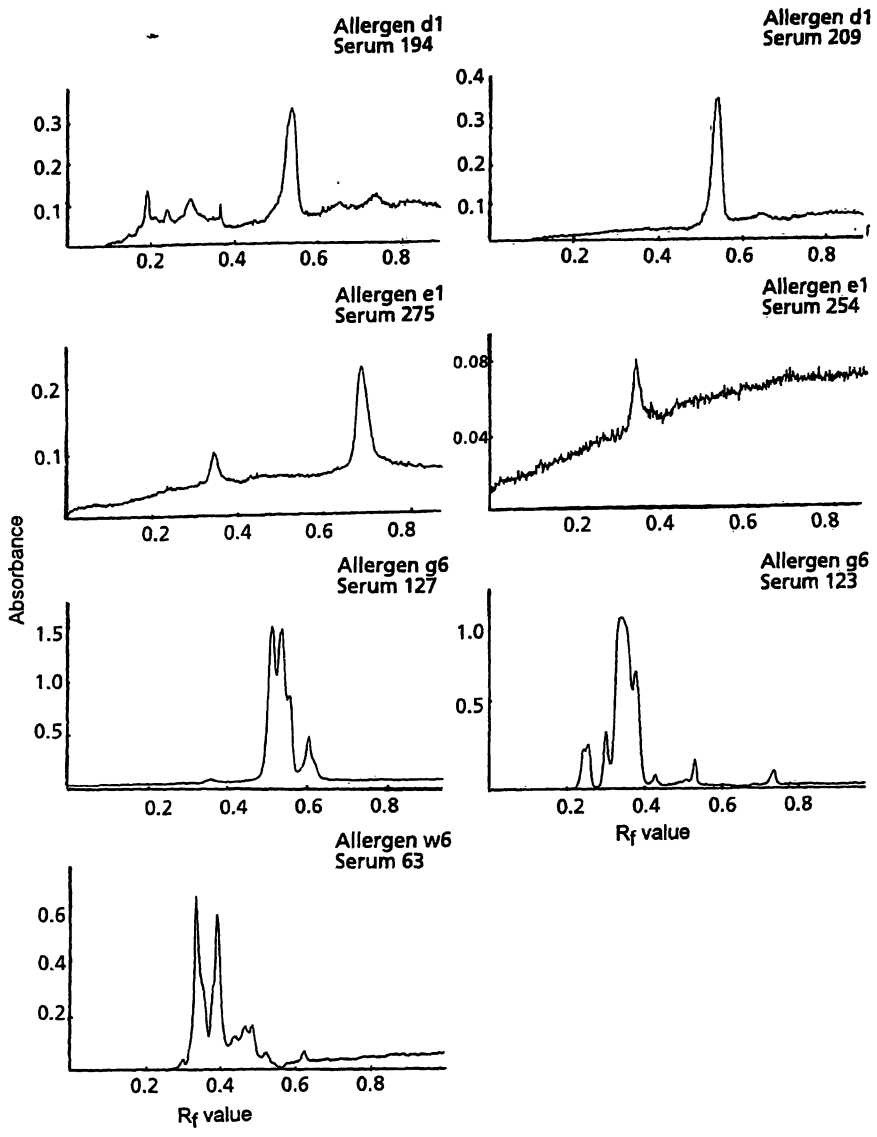

Fig. 1 Detection of allergen specific IgE antibodies by immunoblotting with pollen (timothy (*Phleum pratense*) and mugwort (*Arthemisia vulgaris*)), house dust mite, cat dander in serum samples with discordant test results. (Pharmacia+/DPC−)

were included. From the precision experiment, with g6 excluded for the DPC Microplate System (above range), the expected standard deviation of the scatter expressed in ln (concentration) was estimated as 0.22. No indication of dependence between differences and concentration level was obtained and the mean difference expressed in per cent of the result with the Pharmacia CAP System was calculated. These values and the CSD-values (the coefficient of sample-related disagreement) are given in table 5. For all allergens the sample-related differences are significant and as can be seen in table 5 the CSD-values are substantially greater than 1.5. This indicates that the error contribution from sample-related differences far exceeds the analytical variation presented in table 2.

Although an xy-plot may indicate a correlation between the methods, a ratio plot shows that the disagreement is considerable. From the ratio plot for d1 for example, it is evident that the ratio y/x varies between 10 and 300%. The sample-related differences are probably caused by different capabilities of the two test systems to measure individual mixtures of IgE antibodies against the allergenic components of an allergen. Furthermore, as the observed mean differences of the DPC Microplate System vary between −25% for g6 and +110% for e5, differences between the allergens also in terms of calibration can hardly be excluded. On the contrary, it is very likely that a variable calibration error is introduced in a system where each individual allergen-reagent lot must be adjusted to an optimal signal level (DPC) rather than to allergen excess (Pharmacia).

It is obvious from the study of systematic differences that several samples with clearly measurable levels of IgE antibodies remain undetected by the DPC Microplate System. This fact and the observation that many cases with low to moderate levels show lower levels with DPC, is in concordance with the sample related differences between methods discussed above. Insuffi-

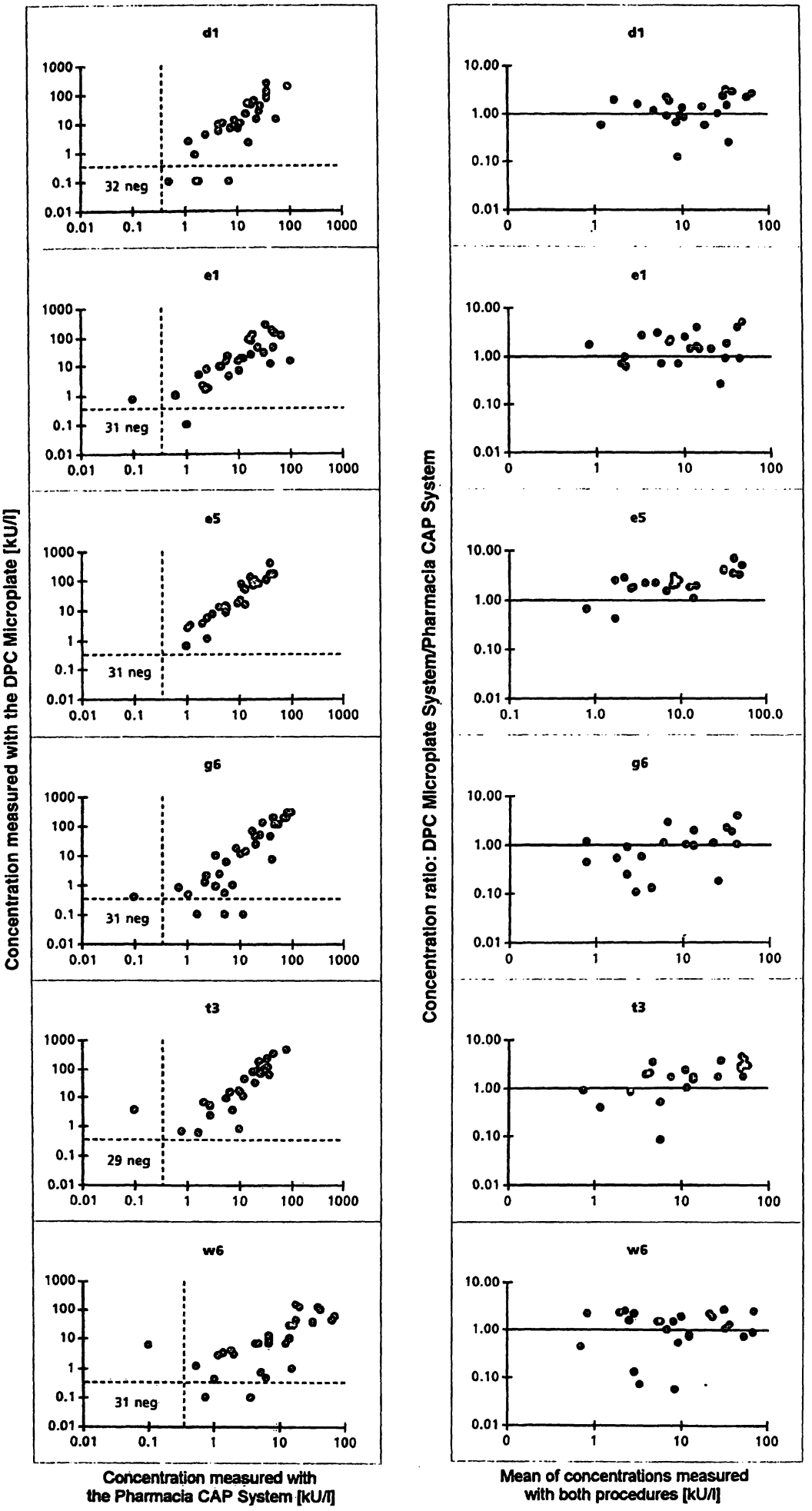

Fig. 2 Concentration with DPC Microplate System (y) is plotted against concentration with Pharmacia CAP System (x) in the diagrams to the left. For results within the measurement range to ratio y/x is plotted against (x + y)/2 in the diagrams to the right.

Tab. 4 Method comparison according to *Passing & Bablok*

Allergen	n	Slope	Intercept	Coefficient of correlation r
d1	26	1.849	−3.089	0.655
e1	24	1.559	−1.421	0.516
e5	23	3.512	−4.630	0.876
g6	22	1.200	−2.076	0.595
t3	20	2.906	−6.257	0.877
w6	27	1.256	0.426	0.809

Tab. 5 Comparison of systematic differences. The mean differences are expressed in per cent of the results with Pharmacia CAP System. CSD is the quotient between the standard deviation of sample-related differences and the standard deviation from the total within run variation.

Allergen	No. of sera	Mean difference %	CSD
d1	22	11	3.23
e1	22	39	3.14
e5	24	110	2.05
g6	19	−25	3.82
t3	22	51	3.32
w6	25	−6	4.09

cient excess of allergen and/or missing allergenic components in the DPC system reagents are the most probable causes for such a deviation.

Effect of dilution

The ratios between observed (O) and expected (E) values for all diluted samples were calculated. Plots of O/E against dilution give a rather regular pattern for the Pharmacia CAP System while the DPC Microplate System exhibits more dramatic effects and very irregular patterns as illustrated in figure 3. Due to the irregular pattern shown by DPC it is not relevant to fit a model to the relationship between O/E and dilution and the results can hardly be summarized numerically in any meaningful way. Dilution of the calibrator 100 kU/l also showed an irregular pattern, which was confirmed in a repeated experiment. The unpredictable result may, according to the manufacturer, be an effect of interference by auto-anti-IgE, affinity dependent variation in a non-excess situation or an under representation of rare allergenic proteins in the allergen reagent.

Effect of addition of unspecific IgE

All values are below the measuring range, i. e. < 0.35 kU/l. Thus, addition of unspecific IgE up to 3000 kU/l does not interfere with specific IgE measurements in the systems under study.

Effect of addition of competing IgG antibodies

The values obtained for sera mixed with negative rabbit serum are referred to as expected values (E) and the observations of all other mixtures (O) are expressed as O/E. The mixtures with negative patient serum are used to check whether dilutions with negative patient and rabbit serum respectively can be considered as equivalent. The obtained values of O/E for these mixtures are in the range 86−107% for Pharmacia and 85−120% for DPC, i. e. well within the range of random variation. For the mixtures with rabbit serum O/E-values are plotted against percent of rabbit IgG serum in the sample. As can be seen from figure 4, the Pharmacia CAP System gives a moderate decrease for increasing amounts of rabbit IgG while the effects in the DPC Microplate System are varying and more dramatic. The results may be explained by the fact that liquid allergens can be aggregated by allergen specific IgG antibodies present in the serum sample (Personal communication, PDC). the data also support the observations made in the evaluation of systematic differences suggesting insufficient allergen excess in the DPC system.

Comparison of calibrators

The results of the DPC calibrators assayed in the Pharmacia CAP System and the Pharmacia calibrators assayed in the DPC Microplate System are shown in table 6. The table shows the observed concentrations in percent of the assigned (expected) concentrations.

The calibrators are in good agreement, and the diverging results obtained when measuring with the DPC Microplate System are in accordance with the high variation seen in the precision study.

User friendliness

The instruments included with the Pharmacia CAP System are MasterCAP, RoboCAP, AutoCAP and Fluorocounter 96. The DPC Microplate System includes MARK 5 Robotic Pipettor, MAX Automated Plate Processor with a built-in kinetic reader and a computer with MAX software designed for DPC Microplate System.

These two systems are similar in function and do not deviate much from a handling point of view, however, the limitations in DPC MARK V, such as the restriction to 4 replicates per patient and the limitation of 96 samples per assay make RoboCAP, where no such limitations exist, a preferable alternative.

One factor we consider negative is the handling of allergens in DPC Microplate System. The allergens supplied in a bottle have to be opened and closed in combination

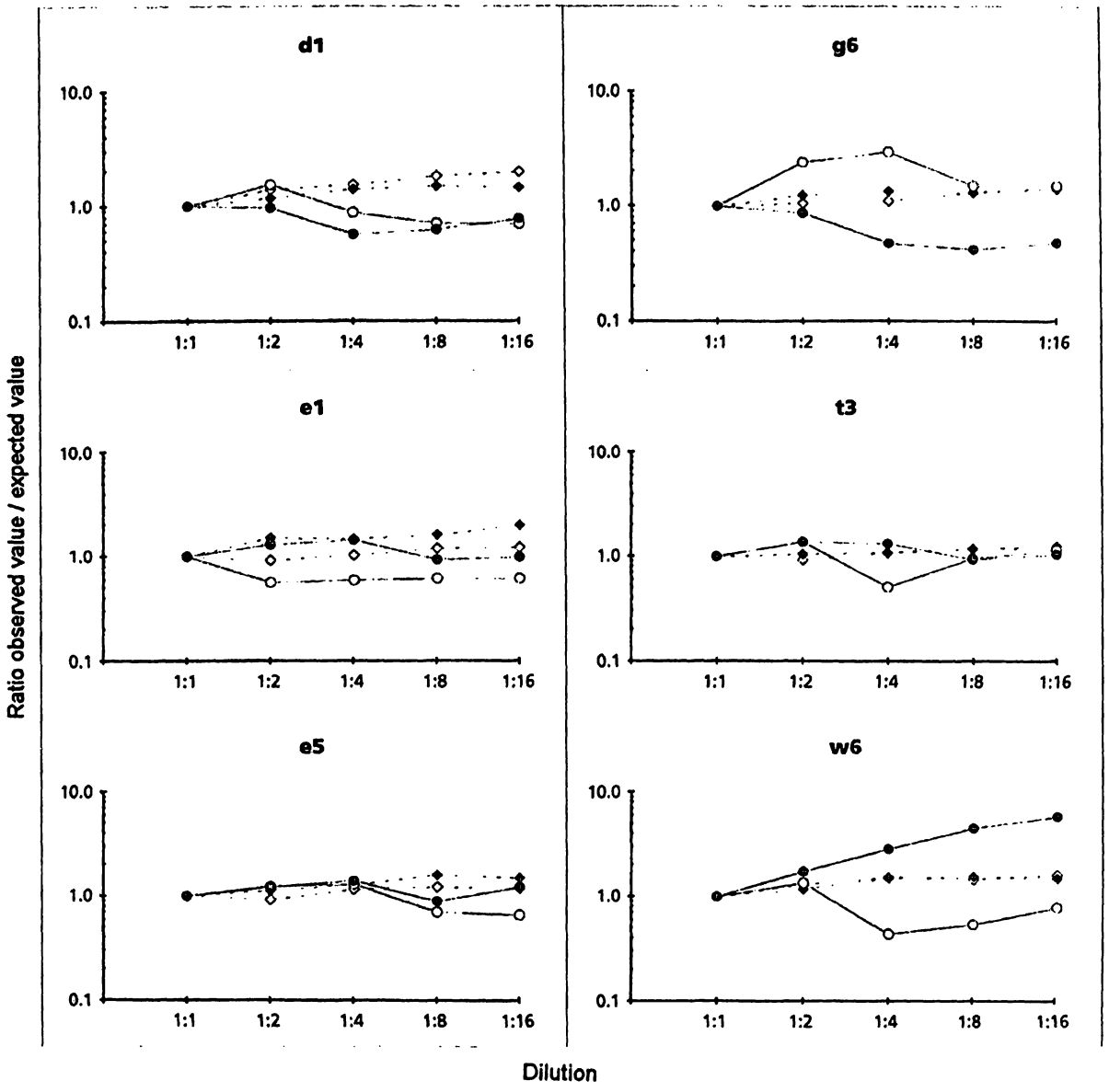

Fig. 3 Results from dilutions of two sera (1 and 2) for each allergen with the Pharmacia CAP System (Ph) and the DPC Microplate System (DPC).
O/E plotted against dilution.
---◇--- PH : 1 ——○—— DPC : 1
---◆--- PH : 2 ——●—— DPC : 2

with each assay, a tedious procedure for a large panel of allergens.

For DPC IMMULITE, the first results were obtained after 1 h 15 minutes, the time for 50 results was 2 h 7 minutes. This shows a theoretical time advantage when using the system for differential testing but in practice no advantage in using the DPC IMMULITE AlaTOP for allergy screening instead of the Pharmacia CAP System Phadiatop was found.

RoboCAP is the more flexible and easier to operate instrument when comparing the two systems or system combinations, Pharmacia CAP System to DPC Microplate System and IMMULITE, we find Pharmacia CAP System to be easier to handle as well as to adapt to sample and information flow in our laboratory.

Conclusions

We found no advantages to the combination of two DPC systems from a handling and work flow point of view. Moreover the Pharmacia CAP System is easier to work with than the DPC Microplate System. Comparing Phadiatop and AlaTOP and investigating the specific IgE antibody assays and their capability to meet our quality demands and the need of quantitative measurement expressed by clinicians, we conclude the following:

Phadiatop has a slightly higher sensitivity than AlaTOP either on IMMULITE or on DPC Microplate System. Both tests perform as expected from the literature (5–7). But from a screening point of view high sensitivity is preferential.

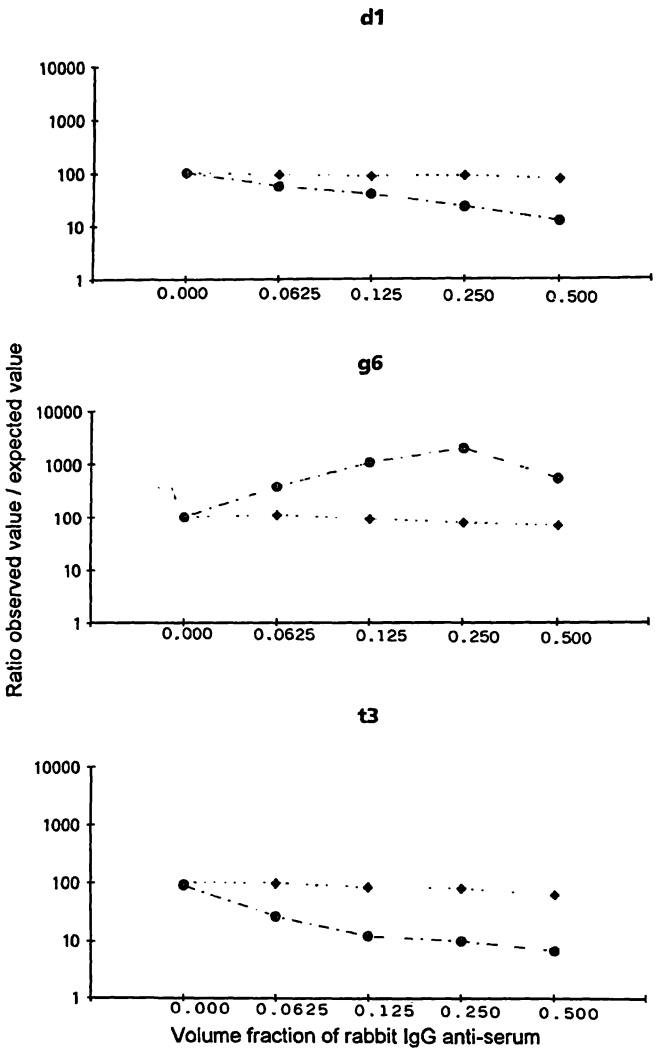

Fig. 4 Effect of increasing amount of rabbit IgG antibodies on the determination of specific IgE against d1, g6 and t3.
····◇···· Pharmacia CAP System
—·—·◇—·— DPC Microplate System.

The DPC Microplate System was shown to be less accurate when compared with the Pharmacia CAP System. This is true for moderate and higher concentrations, where we found 2.5 times higher CV's. But at concentrations below 3.5 kU/l the DPC Microplate System shows, in general, higher CV's. The comparison of results show that the two methods do not, to the full extent, measure the same antibodies. This can be concluded based on the results of the correlation/regression (*Passing, Bablok*) but more clearly based the study of the sample related disagreement (CSD).

Furthermore DPC failed to detect specific IgE in at least 12 out of 187 patient samples which were in 8 cases found positive with Pharmacia CAP System and confirmed with blotting experiments. In contrary only 2 samples that were positive by DPC Microplate System and confirmed by blotting were found negative in Pharmacia CAP System. 35 results out of the 187 patient samples were above the calibrator range in the DPC Microplate System. The corresponding figure for the Pharmacia CAP System was 1 result.

There was no effect when adding non-specific IgE to serum samples and the comparison of calibrators show a good agreement. When looking at the addition of competing IgG antibodies we demonstrated a moderate decrease in binding of specific IgE in Pharmacia CAP System.

The effect in the DPC Microplate System was more pronounced with large decreases or increases of measured values even at lower concentrations. The results may indicate insufficient allergen concentration in the DPC assay and draw attention to the risk for undesirable complex formation between allergen and antibody in solution.

Finally we conclude that the Pharmacia CAP System provides quantitative measurement of allergen specific IgE antibodies with acceptable analytical variation (Pooled CV ≈ 10%), whereas the DPC Microplate Sys-

Tab. 6 Observed concentrations of calibrators in percent of the assigned concentrations.
* = value obtained by extrapolation.

DPC calibrators assayed in Pharmacia CAP System Concentration (kU/l)		O/E × 100 (%)	Pharmacia calibrators assayed in DPC Microplate System Concentration (kU/l)		O/E × 100 (%)
Assigned	Observed		Assigned	Observed	
0.35	Below range	–	0.35	0.33**	93
0.7	0.79	112	0.7	0.70	100
3.5	3.49	100	3.5	4.04	115
17.5	17.8	101	17.5	17.9	102
52.5	50.2	96	50	61.9	124
100	Above range	–	100	94.6	95

tem results in higher variation and unwanted side effects when diluting and interfering with IgG. There also seem to be some problems with stability of the specific IgE test. The data obtained in this study support the statement that allergen excess is needed for accurate measurement of IgE antibodies. Even though the reagent prices are marginally higher, we prefer the Pharmacia CAP System for differential as well as specific IgE testing.

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